

Evidence of spermatophores in Cyphophthalmi (Arachnida, Opiliones)

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- A spermatophore in Opiliones is for the first time observed and described from a specimen of *Cyphophthalmus serbicus* (Hadži, 1973), here transferred from *Siro*. Insemination by means of spermatophores seems typical for some groups of the Cyphophthalmi, the most primitive and the least species-rich suborder in the order Opiliones. Primitive but complex insemination by spermatophores was retained in this group, which is adapted to a cryptozoic way of life, where this mating strategy is still functional. This paper presents a hypothesis on the possible significance of a number of characteristic traits in Cyphophthalmi, which presumably have some function for insemination via spermatophores.

Keywords: Opiliones - Cyphophthalmi - spermatophores - sperm transfer - functional anatomy - Balkan.

INTRODUCTION

The Cyphophthalmi is a rare and not well studied group of small cryptobiotic animals. They are present on almost all continents, with discontinuous distribution patterns and high degrees of endemism and with 113 known species (Giribet, 2000). Although Juberthie (1960, 1961, 1965, 1967) explained many aspects of biology and anatomy of Cyphophthalmi using *Siro rubens* Latreille, 1804 as an example, function and significance of many specific characters are still not understood. The presence of penis and ovipositor, and copulation as a direct way of insemination are used as differential characteristics of the order Opiliones (Martens, 1976). Dogmatic influence of this widely accepted concept led numerous authors to neglect the assumption that insemination in Cyphophthalmi is through deposition of spermatophores, which could in turn explain the function of certain characters.

First speculations on spermatophore production in Cyphophthalmi were presented by Forster in his thesis (*vide* Juberthie, 1965; Savory, 1977; Shear, 1980). Forster (1948, 1952) conducted extensive and detailed faunistic and taxonomic research on Cyphophthalmi in New Zealand and was able to notice this extremely important trait. Unfortunately, Forster's observations and assumptions were not

accepted, with other authors being skeptical (Juberthie, 1965; Shear, 1980), or ignoring them entirely. However, the possibility that Cyphophthalmi might indeed form spermatophores was not ruled out (Juberthie & Manier, 1978; Martens, 1978). Regarding the penis anatomy in Cyphophthalmi, van der Hammen (1985) assumed its function to be deposition of spermatophores and used the appropriate term, spermatopositor. However, he did not give any further explanation and it can be assumed that this was the manifestation of adopting Forster's assumption. In his attempts to separate the Cyphophthalmi from the other Opiliones, Savory (1977) was the only one to point out the importance of Forster's findings and used them as a unique key trait of Cyphophthalmi to support his ideas (Savory's hypothesis was that Cyphophthalmi is an ancestral group of the remaining Opiliones and of the Ricinulei, and he suggested that they comprise an order of their own; this has been refused by other authors). With the exception of some differential traits that comprise a complex reproductive mechanism, there are indeed few characters which are not essentially opilionid characters (van der Hammen, 1985). In that respect, this paper has no ambition to discuss speculations about the phylogenetic position of Cyphophthalmi.

Studying and collecting Balkan sironids for a number of years led to the discovery of an important trait which supports Forster's assumption that sperm transfer in Cyphophthalmi is accomplished by means of spermatophores. In contrast to Forster, who had circumstantial evidence (Shear, 1980) and only assumed the existence of spermatophores, my evidence is an actual spermatophore attached to a female specimen. Except for the morphological description of the spermatophore (no histological details are given since this is the only complete sample available to date) and its attachment to an ovipositor, the rest of this paper is of speculative nature, relying on available facts and logical reasoning. Researchers collecting Cyphophthalmi know how hard it is to find them, while the collecting technique itself (soil and litter sieving) further significantly decreases the probability of finding a female with an attached spermatophore. After twenty years of research and with over 1000 specimens collected, only two females have been found with spermatophores attached. Therefore, and in spite of the scarcity of information, I have decided to publish these findings.

MATERIAL AND METHODS

Material: 1 female *Cyphophthalmus serbicus* (Hadži, 1973) **comb. n.**¹: Serbia, Svrlijske planine Mts., above village Crnoljevica 14.07.1989, leg. I. Karaman.

1 female *Cyphophthalmus* sp. 1.: Serbia, Mt. Zlatibor, Šargan, 14.06.1991, leg. I. Karaman.

11 females of *Cyphophthalmus* sp. 2.: Montenegro, Danilovgrad, Milovička vrela, 26.04.1997, leg. I. Karaman.

Methods: The material was preserved in 70% ethanol. Details of the spermatophore were observed and photographed in glycerine (LM microphotographs). SEM

¹ All known Balkan Cyphophthalmi belong to one genus (resurrected genus *Cyphophthalmus* Joseph, 1868), which is not closely related to the genus *Siro* Latreille, 1796 (Karaman, in prep.). To avoid possible later confusion, I decided to use the correct generic name for the species included in this paper.

photographs of material prepared in a Baltec SCD005 Sputter Coater (ovipositors where frozen before) were made with a JEOL-JSM-6460lv SEM microscope in high vacuum.

RESULTS AND DISCUSSION

Figure 1 shows a female of *C. serbicus* with an attached spermatophore as it was found in my collection where the specimen was kept for more than 10 years. The spermatophore is balloon-like in shape (Fig. 2A) and extends into a tube which ends freely in an amorphous mass that is well attached to the basal parts of the terminal lobes of the ovipositor. Entrances to receptacula seminis are widely opened (Fig. 2C). Figure 2B illustrates how the spermatophore is attached to the female ovipositor in the specimen of *Cyphophthalmus* sp. 1. Not understanding its importance in my earlier studies, the spermatophore on this specimen was regrettably cut off and lost later on. Specimens collected on the same occasion were my first finds of a new species of Cyphophthalmi, and thus the importance of this detail was overlooked. The tubular appendage, however, remained attached to the ovipositor. Interestingly, the tubular parts of spermatophore in *C. serbicus* and *Cyphophthalmus* sp. 1 differ considerably. The external layer of the spermatophore tube in *Cyphophthalmus* sp. 1 is twice as wide and more transparent (almost membrane-like) than in *C. serbicus*, where it is amber in colour and appears brittle (Fig. 4A, C) (terminal part missing probably due to this fragility). These two species are phylogenetically distant (based on yet unpublished data).

A globular structure is visible inside the spermatophore (Fig. 4B), with spherical contents which likely are encapsulated sperm. The dimensions of the individual spheres (30-45 μm) are in accordance with the encapsulated sperm described by Juberthie (1965). However, the diameter of the spermatophore tube is 9 μm (Fig. 4C), which is too narrow for the encapsulated sperm to pass through, nor can the opening of the receptaculum seminis ($\approx 15 \mu\text{m}$) receive such encapsulated sperm cells. Therefore I suppose that females exert mechanical pressure on spermatophores, causing the capsules to break and release the sperm that can then pass through the tube easily (they are in fact pushed out). This assumption is also supported by the fact that the basal end of the spermatophore is depressed (Figs 1, 2A, 4).

The receptacula seminis may also play an active role in receiving the released spermatophore contents, with the muscles at the basal end of the receptacula possibly having such a function. These muscles were mentioned and illustrated for the first time by Rafalski (1958) in *Siro carpaticus* Rafalski, 1958. The same muscles were shown in an illustration of the ovipositor longitudinal section of *Siro duricorius* Joseph, 1868 in Martens *et al.* (1981), but no explanation was given. The active function of the receptacula seminis in sperm reception might be another specific characteristic of the Cyphophthalmi.

The above-described way of sperm transfer requires certain anatomic adaptations (specific for Cyphophthalmi). The position of the ovipositor during sperm transfer is characteristic, with the apical part obliquely extended and set in the groove of the posterior edge of the genital opening (Fig. 2B). The posterior edge of the genital opening is itself protruded outward, enabling the oblique position of the ovipositor

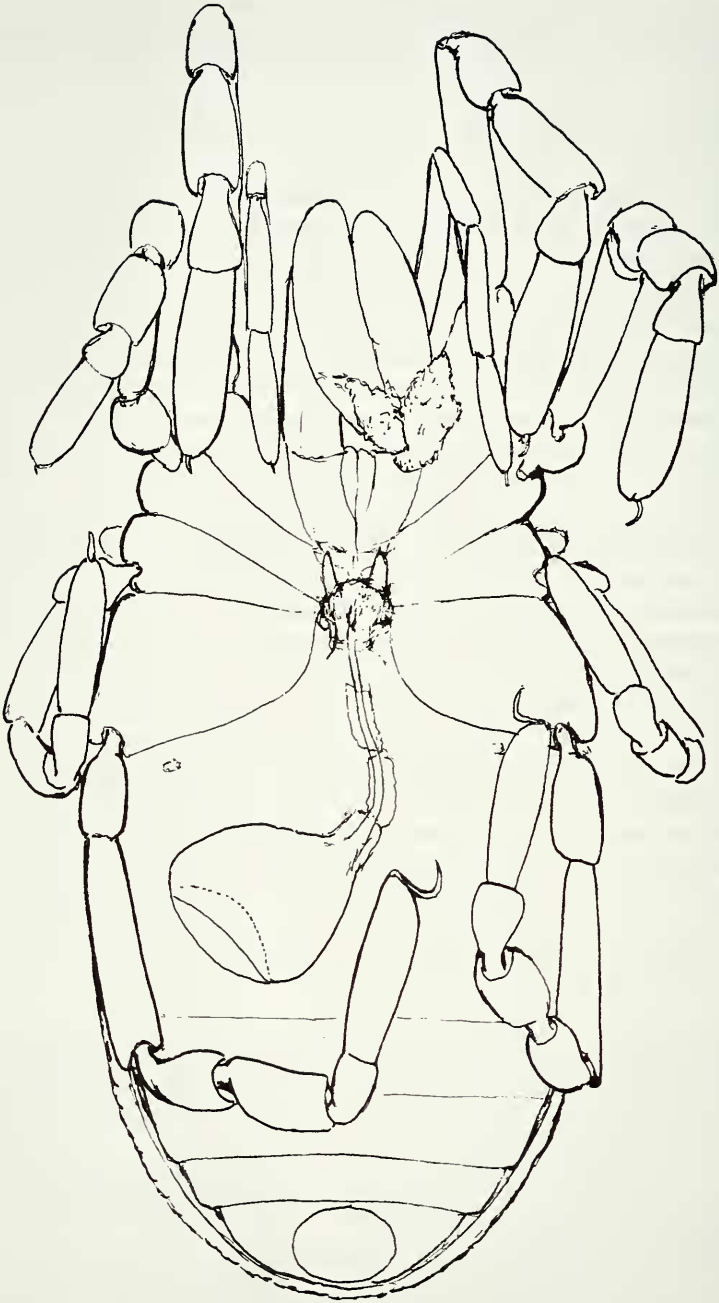


FIG. 1

A female of *Cyphophthalmus serbicus* with attached spermatophore, ventral view.

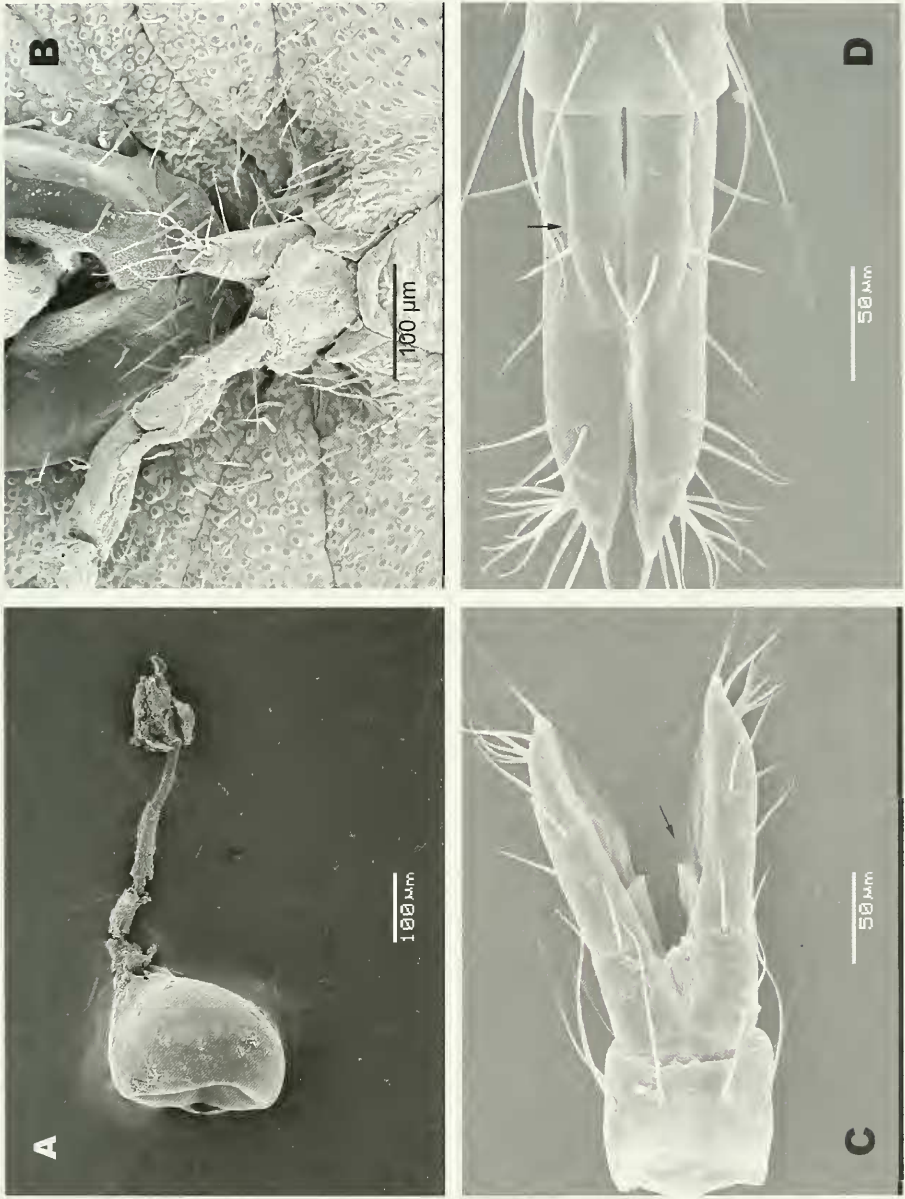


FIG. 2

A. Spermatophore of *Cyphophthalmus serbicus*. B. Terminal part of spermatophore of *Cyphophthalmus* sp. 1 attached in between terminal lobi of ovipositor. C. Terminal part of ovipositor of *C. serbicus* after retraction of the spermatophore, with widely opened entrance to receptacles (arrow). D. Ventral view on terminal part of ovipositor of *C. serbicus* (arrow marks swollen area of terminal lobus).

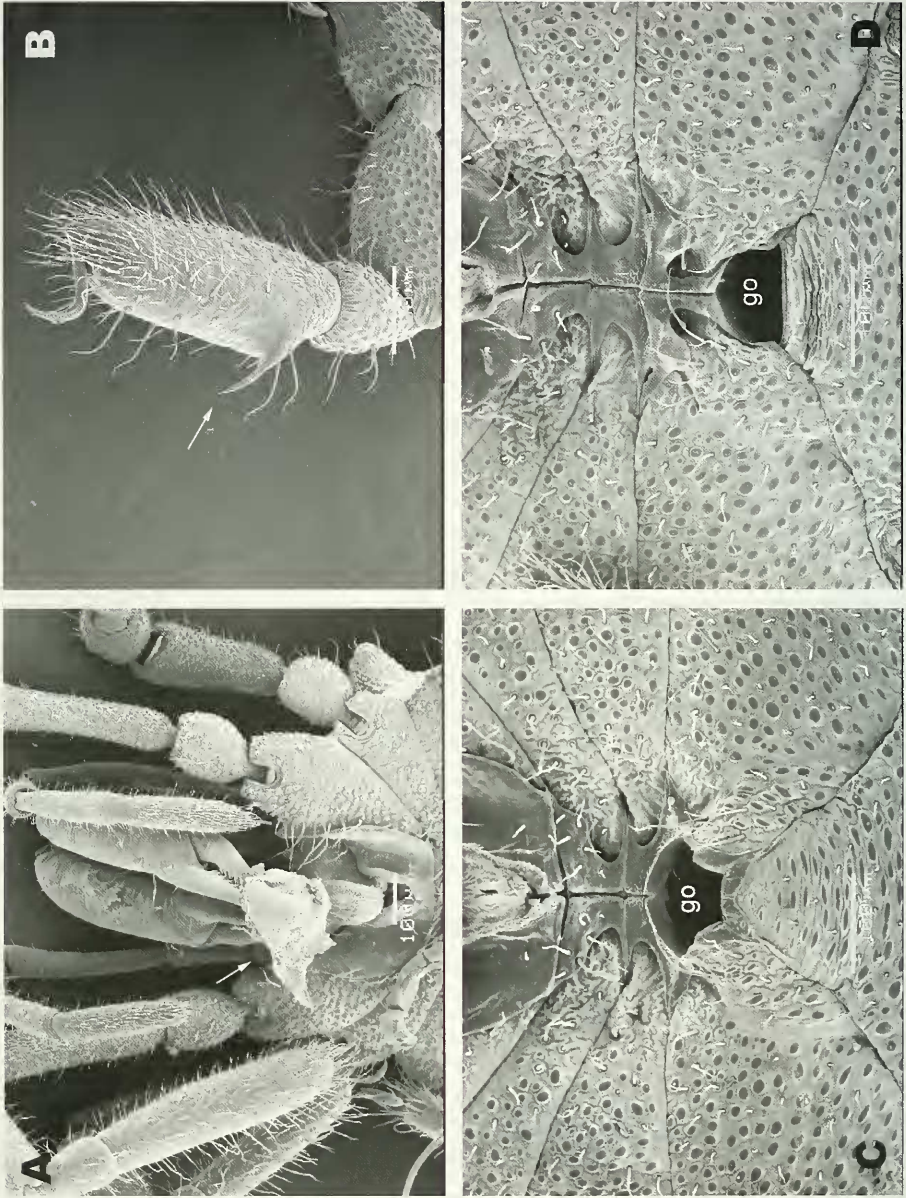


FIG. 3

A. Terminal parts of chela of *Cypho-phthalminus* sp. 1 with amorphous mass (arrow), supposed to be the secretion of the ovi-positor sticky glands. B. Male tarsus IV with post-tetradorsal adenostyle (arrow) terminally bearing opening of the tarsal gland. C. *Cypho-phthalminus serbicus* female, genital opening (go) with coxosternal complex anterior to it. D. Genital opening with coxosternal complex of a male of *C. serbicus*.

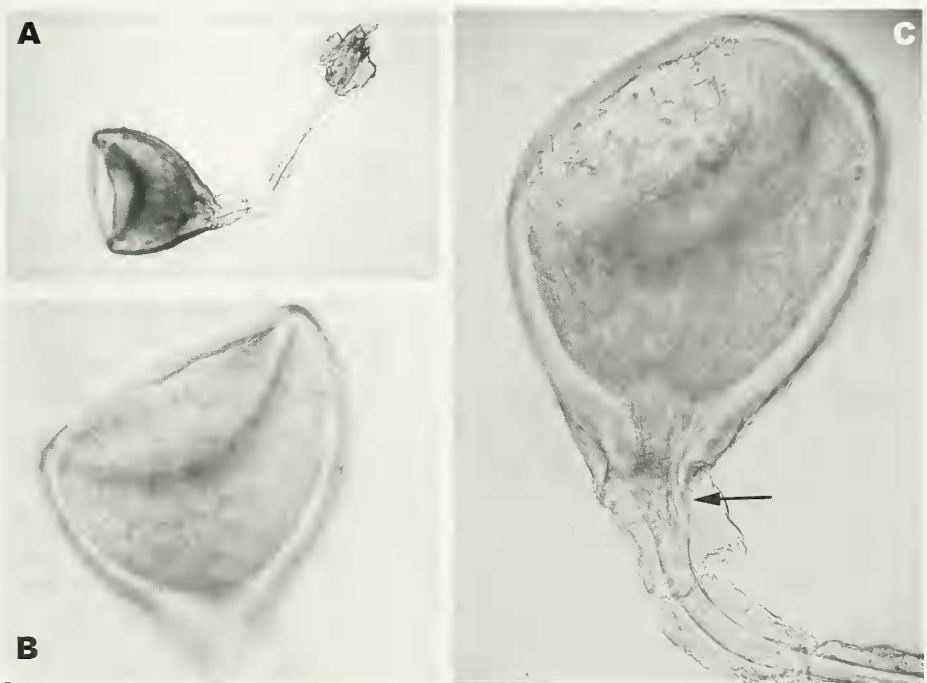


FIG. 4

A. LM microphotograph of the whole spermatophore of *Cyphophthalmus serbicus*. B. Main portion of spermatophore showing globular structure of its contents. C. Basal part of spermatophore tubular appendage with a content which could represent released sperm cells (arrow).

during sperm transfer (Figs. 2B, 3C). The ventral side of the terminal lobes of the ovipositor is "swollen" in the basal area (causing asymmetry in the frontal plane) (Fig. 2D), at the point where the end of the spermatophore is attached to the ovipositor. Likely this creates space for the spermatophore to attach and for sperm transfer.

The whitish amorphous mass that connects the terminal end of the spermatophore and the ovipositor lobes might be a secretion of the sticky glands ("Kittdrüse") placed in the terminal lobes of the ovipositor (Martens *et al.*, 1981). It is interesting to mention that a similar mass covers the tips of the chela in both specimens (Figs 1, 3A). Since nothing similar was observed in any of the other specimens in my collection, it is possible that the presence of this secretion on the chelicerae is somehow connected with the process of spermatophore transfer (manipulations for placing the terminal part of the spermatophore in the adequate position between the ovipositor lobes).

The lack of a genital operculum in both sexes is another character of *Cyphophthalmi* that might be explained by the specific way of insemination. The operculum would obstruct the complex process of spermatophore formation and transfer of its contents (for males and females).

The structure of spermatophore and its external layer suggest that there is no copula in the genus *Cyphophthalmus*. I believe that the primitive type of mating, by deposition of spermatophores without previous contact, was retained in this genus.

Encapsulated sperm and well-protected spermatophore imply that a male can lay the spermatophore on the ground even if a female is not present. Another specific character of some Cyphophthalmi is the presence of anal glands in males. The secretion of these glands by males of the genus *Cyphophthalmus* can be used to mark the place where a spermatophore has been deposited in order to attract females.

An active role of the ovipositor in receiving and maybe even finding spermatophores is also possible.

The fact that Juberthie never noticed spermatophores while studying the biology of *S. rubens* could be explained by the camouflage colour of spermatophores, which is yellowish amber in the specimen of *C. serbicus* in my collection. Spermatophores with such a colour are inconspicuous when on the ground.

The formation of the spermatophore is possibly supported by two annex glands that open in the lumen of the terminal end of the vas deferens (Juberthie, 1965). The thick external layer of the spermatophore could be the product of the gland on tarsus IV of males (Fig. 3B) (this could be its primary function). The complex structure of the voluminous tarsal glandular organ (Martens, 1979) supports this assumption.

It would be interesting to determine the exact location where spermatophores are formed. This could take place on the surface of the coxosternal complex of the male, and it is possible that this complex plays some role in the process of spermatophore production. The coxosternal complex shows distinct sexual dimorphism in Cyphophthalmi (Figs 3C, D), which is not expressed quite so distinctly in any other group of Opiliones. The coxosternal complex is largely reduced in females, while it is well-developed in males, and it is often species specific. In males this is a structured smooth surface covering the bottom of the depression in front of the genital opening, and the only such formation in its proximity. This depression lies immediately below the terminal end of the spermatopositor when it is extended.

As expected for endogean sironids from a temperate region, seasonality in mating was observed. The time of mating varies depending on latitude, altitude and exposition, and it may be species specific too. At one locality in southern Montenegro (50 m asl) specimens of a still undescribed species (*Cyphophthalmus* sp. 2) were mating at the end of April. This conclusion is based on the fact that about 25% of all collected females (45 specimens) had extended ovipositors with an amorphous mass between their terminal lobes, possibly the secretion from sticky glands in the terminal lobes of the ovipositor. It seems that specimens were caught during sperm transfer. In west Serbia and at 800 m asl a specimen of *Cyphophthalmus* sp. 1 was found with a spermatophore in the middle of June. The female of *C. serbicus* with a spermatophore attached to it was collected in the middle of July on the northern slopes of the Svrlijske planine Mountains (east Serbia), at altitudes higher than 800 m asl.

This primitive but very complex insemination mechanism, considered as a plesiomorphic trait, was retained by some Cyphophthalmi. It is a small group of opiliones, specialized for a cryptozoic way of life in small crevices, holes and similar microhabitats where this mating strategy can remain functional. It is clear that other Opiliones have acquired a direct way of insemination, i.e. the spermatopositor has developed into a penis. However, I believe that some form of transitional insemination (direct transfer of spermatophores or sperm packets) can be found in some Opiliones.

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